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Short Communication

Genomic Organization of Mouse and Human GTP Cyclohydrolase I Genes and Mutations Found in the Human Gene

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Introduction

GTP cyclohydrolase I (EC 3,5,4,16) is the first and rate-limiting enzyme for the biosynthesis of tetrahydrobiopterin in mammals (1). Tetrahydrobiopterin has multiple physiological functions. It acts as an essential cofactor for three aromatic amino acid monooxygenases, i.e., phenylalanine, tyrosine, and tryptophan hydroxylases (2-4). These enzymes are essential for synthesizing hormones and neurotransmitters such as dopamine, noradrenaline, adrenaline, and serotonin. It has also been shown that tetrahydrobiopterin acts as a cofactor for the generation of nitric oxide (NO) from arginine (5,6).

GTP cyclohydrolase I cDNA was first isolated from a rat liver cDNA library (7). We had reported the isolation of three types of GTP cyclohydrolase I cDNA clones from human liver (8). The human three cDNAs were identical at their central and 5' regions but diverged at their 3' ends. We designated these three cDNAs as type 1, type 2, and type 3. Type 1 cDNA, which has the longest coding region, consisting of 250 amino acids, corresponds to the cDNA reported from rat (7) and mouse (9).

Very recently we found that the gene for GTP cyclohdrolase I is a causative gene for hereditary progressive dystonia with marked diurnal fluctuation (HPD), also dnown as DOPA responsive dystonia (DRD) (10). This disease is inherited as an autosomal dominant trait with a low penetrance. We found that HPD/DRD patients had genetic defects in the GTP cyclohydrolase I gene only in one allele and that their GTP cyclohydrolase I activity in mononuclear blood cells was reduced to less than 20% of that of normal individuals. If only one allele carries the mutant gene, the enzyme activity would be expected to be decreased to about half of the control value. We assumed that expression of GTP cyclohydrolase I in HPD/DRD patients would be lower than that in normal individuals. To elucidate the etiology of HPD/DRD in more detail, it is important to explore the mechanisms regulating the expression of the GTP cyclohydrolase I gene.

On the other hand, there have been several reports on tetrahydrobiopterin-dependent hyperphenylalaninemia caused by a deficiency in GTP cyclohydrolase I (11-13). Hyperphenylalaninemia caused by GTP cyclohydrolase I deficiency shows more severe symptoms than that caused by a defect in phenylalanine hydroxylase, because a deficiency in GTP cyclohydrolase I impairs the biosynthesis of both catecholamines and serotonin due to the lack of tetrahydrobiopterin. The patients show severe retardation of development, severe muscular hypotonia of the trunk and hypetronia of the extremities, convulasins, and frequent episodes of hyperthermia without infections (11-13).

Here we have reported genormic DNA structures for the human and mouse GTP cyclohydrolase I

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genes and mutations in the GTP cyclohydrolase I gene found in patients with GTP cyclohydrolase I deficiency (14,15).

Materials and Methods

A mouse genmic library, constructed in λ FixII phage vectors (Stratagene), was screened by use of a mouse GTP cyclohydrolase I cDNA clone (9) labeled with [α-^{32}P]dCTP. Human genomic DNA libraries, constructed in Charon4A (donated bv Dr. Tom Maniatis: Harvard University) and in EMBL3 (LI018; donated by the Japanese Cancer Research Resources Bank) were screened with a human GTP cyclohydrolase I cDNA clone (8) used as a probe. DNAs were isolated from the purified phage plaques following standard protocols, and the fragments of interest were subcloned into pBluescriptKS (Stratagene) or pUC 119 vectors for furthe structural analysis.

Genomic DNA was extracted from primary skin fibroblasts. We amplicied exons for GTP cyclohydrolase I including splicing junctions using the PCR on genomic DNA. Primer sequences used for amlification of exons were as follws: exon 1,5'-GTTTAGCCGCAGACCTCGAAGCG-3' and 5'-GAGGCAACTCCGGAAACTTCCTG-3'; exon 2,5'-GTAACGCTCGCTTATGTTGACTGTC-3' and 5'-ACCTGAGATATCAGCAATTGGCAGC-3': exon 3,5' -AGATGTTTTCAAGGTAATACATTGTCG-3' and 5'-TAGATTCTCAGCAGATGAGGGCAG-3'; exon 4,5'-GTCCTTTTTGTTTTATGAGGAAGGC-3' and 5'-GGTGATGCACTCTTATAATCTCAGC-3'; exon 5,5'-GTGTCAGACTCTCAAACTGAGCTC -3' and 5'-TCACTTCTAGTGCACCATTATGACG -3'; enon 6,5'-ACCAAACCAGCAGCTGTCTACTCC-3' and 5'-AATGCTACTGGCAGTACGATCGG-3'. PCR amplification and sequence analysis were performed as described previously (10).

Results

Using the cloned mouse and human cDNAs as a probe, we isolated genomic DNA clones for mouse and human GTP cyclohydrolase I. Structural analysis of these clones and PCR analyses revealed that the both mouse and human genes consist of a total of 6 exons (Fig. 1). We sequenced the exons and their splice sites.Each exon/intron boundary conformed perfectly to the GT/AG rule (16).

The nucleotide sequences of the 5'-flanking region for the mouse and human genes were determined to about 600 bp upstream from the transcriptional starting site. No obvious TATA box was

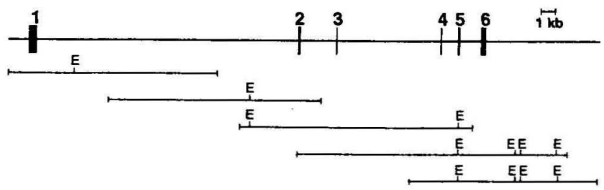

Fig. 1. Structure of the mouse GTP cyclohydrolase I gene. The structure of the mouse GTP cyclohydrolase I gene is depicted at the top of the figure. The 6 exons are indicated by closed boxes, and introns are represented by thin horizontal lines. Five isolated clones covering more than 30 kb of the mouse GTP cyclohydrolase I gene are depicted below the structure of the gene. EcoRI restriction sites (E) are indicated by vertical bars.

observed in the promoter region. In the mouse gene, an AT-rich putative projmoter motif (ATAAAAA) or a CCAAT box was present just upstream or 50 bp upstream from the transcriptional startinhg site, respectively, and an H1-box(17), an IBP-1b (18), anhd a GT-2B (19) binding consensus sequence were found. The AT-rich putative promoter motif and CCAAT box found in the mouse gene vere conserved in the human GTP cyclohydrolase I gene A consensus sequence for the binding of GT-2B (19) was found in the human gene as well as in the mouse gene. The IBP-lb and H1-box consensus sequences found in the mouse gene were not present in the human gene, whereas a leader binding protein-1 (LBP-1;20) binding sequence motif ((A/T) CTGG; −475 to −479), a T-antigen binding motif (−272 to −277), a TGGCA box (−671 to −675), and as SP1 consensus sequence (−236 to −245) were present in the human gene.

To search for mutations in the coding region of the human GTP cyclohydrolase I gene, we amplified exons including splicing junctions from genomic DNA by PCR. Amplified DNA fragments were directly sequenced with an automated DNA sequencer.

We examined two patients with GTP cyclohydrolase I deficiency (Patient N.R. and Patient M.K) and normal individuals. Patients had missense mutations in both alleles. Patient N.R. had an A nucleotide instead of the G nucleotide found in normal subjects, and this mutation resulted in an amino acid substitution of methionine by isoleucine at position 211 (Met211Ile) (14). Patient M.K. showed a transition from G to A, and the arginine residue was substitute with a histidine residue at position 184 (Arg184His). Both patients were homozygous in terms of the mutations, and no other mutations were found in the coding region of their GTP cyclohydrolase I gene. These substiution affect

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human  MEKGPVRAPA EKPRGARCSN GFPERDPPRP GPSRPAEKPP RPEAKSAQPA DGWKGERPRS   60
  rat            M -----V--T- -----EL--- -A------SR P----G---- -A--AG----   51
mouse            M -----V--T- --S--EL--- -A-P----SR P----G---- -A--AG-H--   51

                                      88
human  EEDNELNLPN LAAAYSSILS SLGENPQRQG LLKTPWRAAS AMQFFTKGYQ ETISDVLNDA  120
  rat  ---------- ---------R ----D--=-- ---------T ---------- ----------  111
mouse  --E-QV---K ---------L ----D--=-- ---------T ---Y------ ----------  111

                   134
human  IFDEDHDEMV IVKDIDMFSM CEHHLVPFVG KVHIGYLPNK QVLGLSKLAR IVEIYSRRLQ  180
  rat  ---------- ---=------ ---------- R--------- ---------- ----------  171
mouse  ---------- ---=------ ---------- R--------- ---------- ----------  171

         184                201        211
human  VQERLTKQIA VAITEALRPA GVGVVVEATH MCMVMRGVQK MNSKTVTSTM LGVFREDPKT  240
  rat  ---=------ -------Q-- -----I---- ---------- ---------- ----------  231
mouse  ---=------ -------Q-- -----I---- ---------- ---------- ----------  231

human  REEFLTLIRS                                                        250
  rat  ----------                                                        241
mouse  ----------                                                        241
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Figure 2. Comparison of amino acid sequences of human (8), rat (7), and mouse (9) GTP cyclohydrolase I's. Amino acids identical with those of human GTP cyclohydrolase I are indicated by hyphens. Positions of aberrant GTP cyclohydrolase I residues found in patients with GTP cyclhydrolase I deficiency (14,15) and in patiens with HPD/DRD (10) are emphasized by the larger letter with the position mumber at the top of the human sequence.

highly conserved amino acid residues of GTP cyclohydrolase I (Fig. 2).

Discussion

Here we demonstrated that patients with GTP cyclohydrolase I deficiency were homozygous for a defect in the GTP cyclohydrolase I gene, whereas HPD/DRD patients were heterozygous. In HDP/DRD patients, we had found three missense mutations, Arg88Trp, Asp134Val, and Gly201Glu, and a two-base insertion that shifts the reading frame just after the translational starting methionine (10). We had expressed the mutated enzymes that had the Arg88Trp or Gly201Glu mutation in the *E. coli* expression system (10). Transfection with cDNA prepared from patients with GTP cyclohydrolase I deficiency as well as that from HPD/DRD ones failed to give the increase in the GTP cyclohydrolase I activity seen in the bacteria transfected with the wild-type cDNA, indicating that mutated enzymes had practically no catalytic activity (10.15). These results indicate that there is no qualitative difference between the mutations in GTP cyclohydrolase I deficiency and those in HPD/DRD. Since GTP cyclohydrolase I deficiency is a recessive disease and both alleles of the gene are mutated as demonstrated by this paper, patients with GTP cyclohydrolase I dificiency have no detectable amount of the enzyme activity.

On the other hand, autosomal dominant inheritance with low penetrance is shown in HDP/DRD. Patients with HPD/DRD carry a mutated gene only in one allele, and they have a little but substantial activity of GTP cyclohydrolase I (10). Therefore the difference in the level of the enzyme activity would explain the differing clinical presentation of these disorders, although both mutations of these disorders reduced the activity of mutated proteins to zero. GTP cyclohydrolase I forms dihydroneopterin triphosphate from GTP through a very complex mechanism (1), and the amino acid sequence of the enzyme is highly conserved among different species. Thus various single amino acid substitutions would be expected to result in a loss of the enzyme activity.

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References

1. Nichol CA, Smith GK, Duch DS. Biosynthesis and metabolism of tetrahydrobiopterin and molybdopterin. Ann Rev Biochem 1985: 54: 729-764.
2. Kaufman S. Studies on the mechanism of the enzymatic conversion of phenylalanine to tyrosine. J Biol chem 1959: 234: 2677-2682.
3. Nagatsu T,Levitt M, Udenfriend S. Tyrosine hydroxylase: the initial step in norepinephrine biosynthesis J Biol Chem 1964: 239: 2910-2917.

4. Lovenberg W, Jaquier E, Sjoerdsma A. Tryptophan hydroxylation: measurement in pieal gland, brainstem, and carcinoid tumor. Science 1967: 155: 217-219.
5. Tayeh MA, Marletta MA. Macrophage oxidation of L-argiine to nitric oxide, mitraite, and mitrate. -Tetraydrobiopterin is required as a cofactor-J Biol Chem 1989: 264: 19654-19658.
6. Kwon NS, Nathan CF. Stuehr DJ. Reduced biopterin as a cofactor in the generation of nitrogen oxides by murine macrophages. J Biol Chem 1989: 264:20496-20501.
7. Hatakeyama K, Inoue Y, Harada T, Kagamiyama H. Cloning and sequencing of cDNA encoding rat GTP cyclohydrolase I. -The first enzyme of the tetrahydrobiopterin biosynthetic pathway- J Biol Chem 1991: 266: 765-769.
8. Togari A, Ichinose H, Matsumoto S, Fujita K, Nagatsu T. Multiple mRNA forms of human GTP cyclohydrolase I. Biochem Biophys Res Commun 1992: 187: 359-365.
9. Nomura T, Ichinose H. Sumi-ichinose C, et al. Cloning and sequencing of cDNA encoding mouse GTP cyclohydolase I. Biochem Biophys Res Commun 1993: 191: 523-527.
10. Ichinose H, Ohye T, Takahashi E, et al. Hereditary progresive dystonia with marked diurnal fluctuation caused by mutations in the GTP cyclohydrolase I gene. Nature Genetics 1994: 8: 236-242.
11. Niedrwieser A, Blau N, Wang M, Joller P, Atares M, Cardesa-Garcia J. GTP cyclohydrolase I deficiency, a new enzyme defect causing hyperphenylalaninemia with neopterin, biopterin, dopamine, and serotonin deficiencies and muscular hypotonia. Eur J Pediatr 1984: 141: 208-214.
12. Dhondt JL, Farriaux JP, Boudha A, et al. Neonatal hyperphenylalaninemia presumably caused by guanosin triphosphate-chclohydrolase deficiency. J Pediatr 1985: 106: 954-956.
13. Naylor EW, Ennis D,Davidson AGF, Wong LTK, Applegarth DA, Niederwieser A. Guanosine triphosphate cyclohydrolase I deficiency: Early diagnosis by routine urine pteridine screening. Pediatrics 1987: 79: 374-378.
14. Blau N, Ichinose H, Nagatsu T, Heizmann CW, Zacchello F,Burlina A.A missense mutation in GTP cyclohydrolase I deficiency missed in the newborn screening program. J. Pediatr 1995: 126: 401-406.
15. Ichinose H, Ohye T, Matsuda Y, Hori T, Blau N, Burlina A, Rouse B, Matalon R, Fujta K, Nagatsu T. Characterization of mouse and human GTP cyclohydrolase I genes: Mutations in patients with GTP cyclohydrolase I deficiency. J. Biol. Chem. 1995; 270 : 10062-10071.
16. Bireathnach R, Chambon P. Organization and expression of eucaryotic split genes coding for proteins. Annu Rev. Biochem 1981: 50: 349-383.
17. Dalton S, Wells JRE. A gene-specific promoter element is requird for optimal experssion of the histone H1 gene in S-phase. EMBO J. 1988: 7: 49-56.
18. Bianar MA. Albert S. Baldwin J, Flavell RA, Sharp PA. A gamma-interferoninduced factor that binds the interferon response sequence of the MHC class I gene, H-2Kb. EMBO J. 1989: 8: 1139-1144.
19. Xiao JH, Davidson I, Ferrandon D, et al. One cell-specific and three ubiquitous nuclear proteins bind in vitro to overlapping motifs in the domain B1 of the SV40 enhancer. EMBO J. 1987: 6: 3005-3013.
20. Jones KA, Luciw PA, Duchange N. Structural arrangement of transcription control domains within the 5'-untranslated leader regions of the HIV-1 and HIV-2 promoters. Genees and Development 1988: 2: 1101-1114.